

Antigenic variation in *Renibacterium salmoninarum* p57: functional and diagnostic implications

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R. salmoninarum P57

Why study antigenic variation?

- Vaccines
- Diagnostic assays

P57 associated with virulence

Major protein expressed during infection.

In vitro:

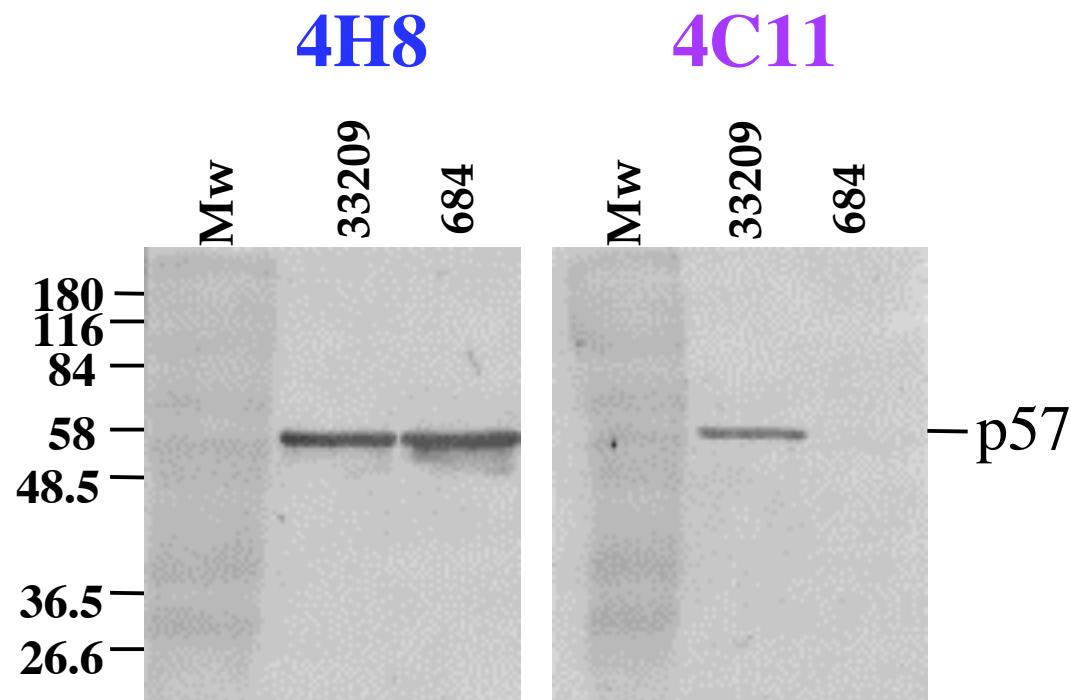
- Binds fish leukocytes and mammalian red blood cells
- Inhibits antibody production of salmonid B cells

In vivo:

- Injection into eggs results in long term immunosuppression
- DNA injection affects immune system molecules
 - Decreases IL1- β , Cox-2, MHC-II
 - Increases TNF α , TGF- β , CCR4, CCR7

Identification of a p57 antigenic variant

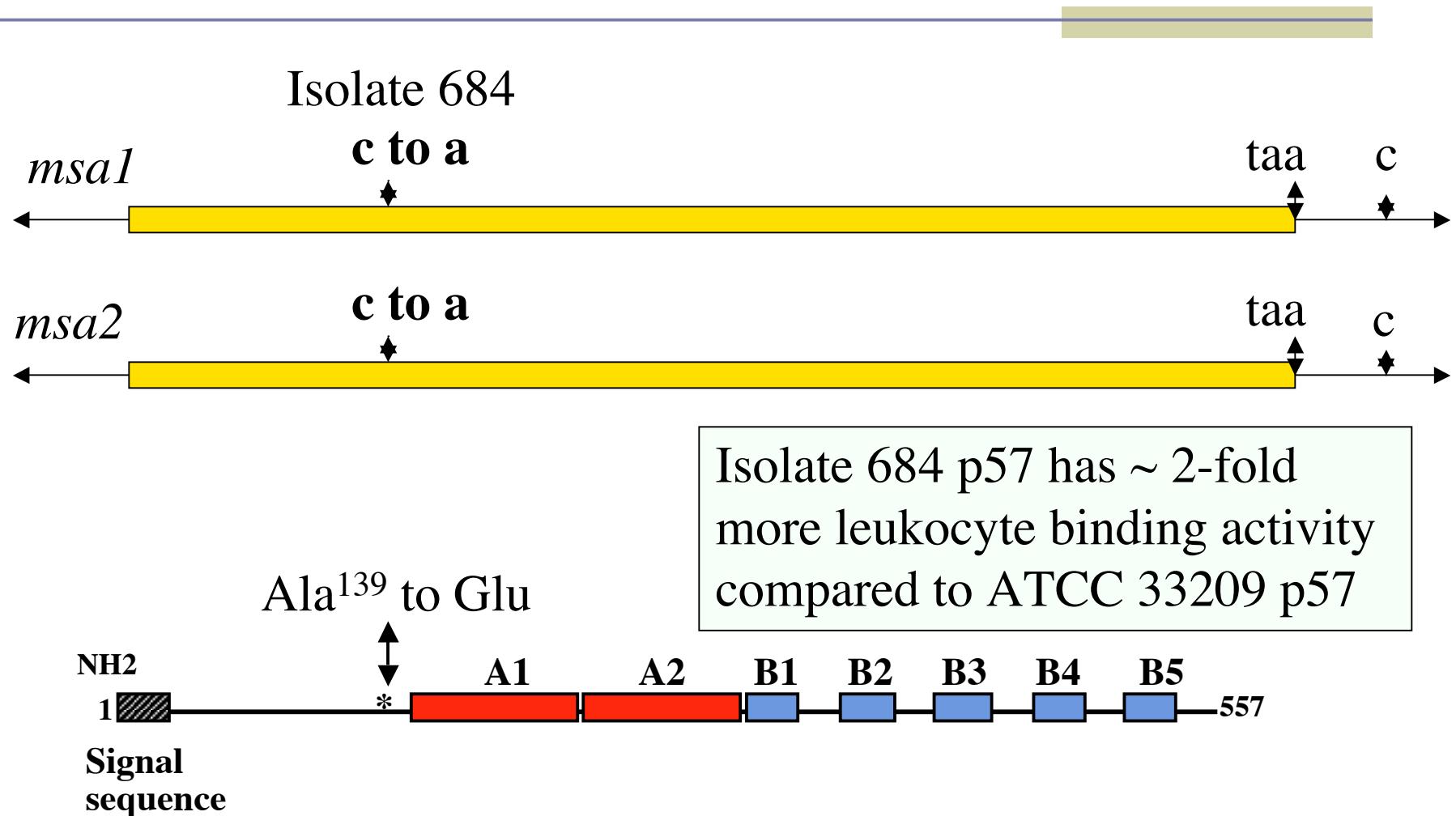
Western blot



684 Strain
Isolated in 1987 from a
Brown trout in Aurland,
Norway

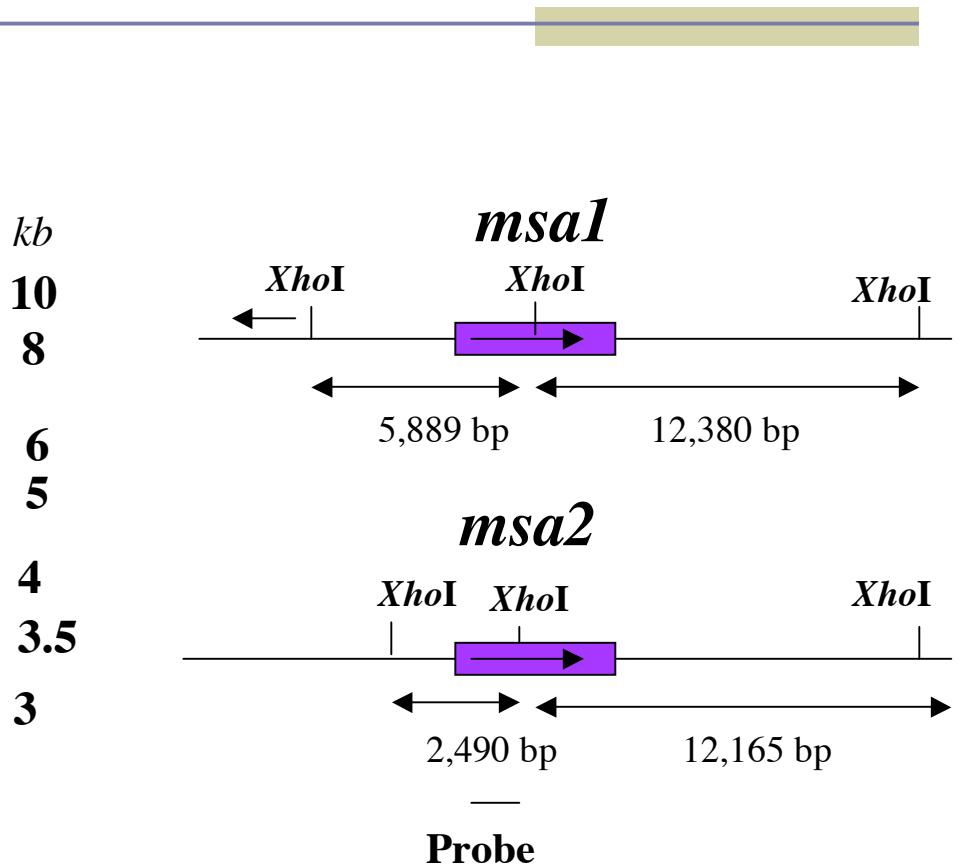
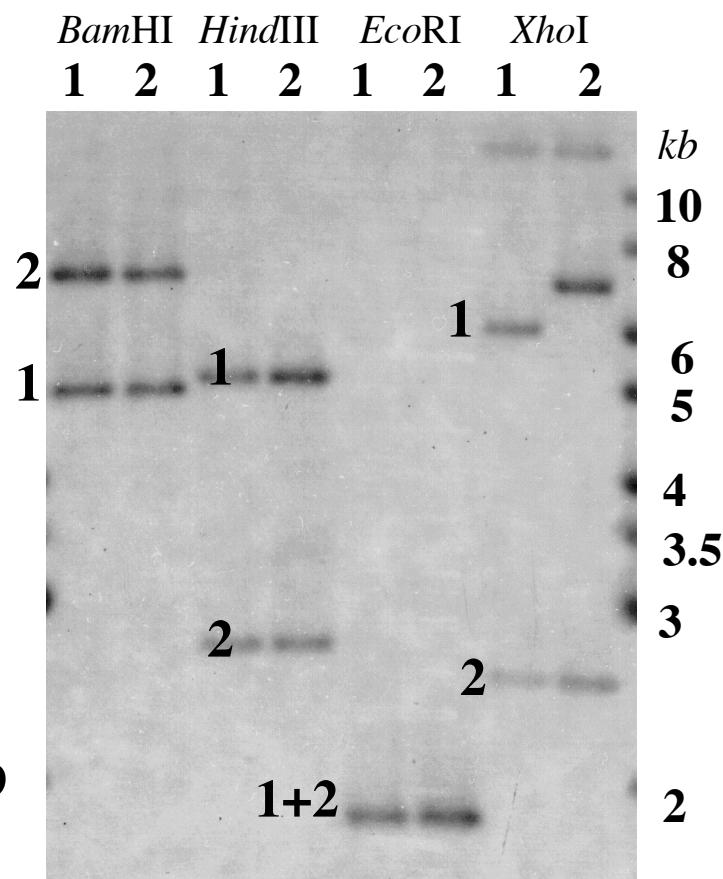
Defect is not in synthesis or
localization of p57

Both *msa1* and *msa2* genes from isolate 684 have identical, single mutations at residue 139.



Wiens, Pascho, and Winton 2002

The location of the Xho I site 5' *msa1* differs between isolates 33209 and 684.



Questions:

- 1. How prevalent is antigenic variation in p57?**
- 2. Is there a correlation between the loss of the 4C11 epitope and the 5'Xho I band shift?**
- 3. Is there a correlation between antigenic variation and other strain markers?**
Exact tandem repeat (ETRA)
16-23S rRNA intervening sequence
- 4. Mechanism of antigenic variation?**

	Isolate	location	Source	Year	Species
1	684/87	Aurland, Norway	O.B. Dale	1987	<i>S. trutta</i>
2	1840/85	Kobbvag, Norway	O.B. Dale	1985	<i>S. salar</i>
3	4451/86	Fjordfisk, Norway	O.B. Dale	1986	<i>S. salar</i>
4	667/85	Fjordlaks Norway	O.B. Dale	1985	<i>S. salar</i>
5	40922/84	Pedro, Norway	O.B. Dale	1984	<i>S. salar</i>
6	WR99C2	Washington?, USA	AquaSeed Co.*	1999	<i>O. kisutch</i>
7	CAR 96	Washington, USA	R. Pascho	1996	<i>O. tshawytscha</i>
8	Man96	Washington, USA	R. Pascho	1996	<i>O. tshawytscha</i>
9	Little Goose	Washington, USA	C. Banner	1984	<i>O. tshawytscha</i>
10	Gr5	Montana, USA	B. MacConnell*	1997	<i>T. thymallus?</i>
11	SAW91	Idaho, USA	R. Pascho	1991	<i>O. tshawytscha</i>
12	DWK90	Idaho, USA	R. Pascho	1990	<i>O. tshawytscha</i>
13	DWK91	Idaho, USA	R. Pascho	1990	<i>O. tshawytscha</i>
14	AK 98	Alaska, USA	DiPac Hatchery*	1998	?
15	BQ96-01	Nanaimo B.C.	J. Ketcheson*	1996	<i>O. tshawytscha</i>
16	BPS91-1	Nanaimo B.C.	J. Ketcheson*	1991	<i>O. gorbuscha</i>
17	DR 143	Nanaimo, B.C.	J. Ketcheson*	1972	<i>S. fontinalis</i>
18	K28	France	B.Austin#	?	?
19	K70	England	B.Austin#	?	?
20	1185/87	Ramsoy, Norway	O.B. Dale	1987	<i>S. salar</i>
21	2649/85	Hatlem, Norway	O.B. Dale	1985	<i>S. salar</i>
22	4467/86	Aurland, Norway	O.B. Dale	1986	?
23	GL64	Lake Michigan	R. Sonstegaard*	1991	<i>O. tshawytscha</i>
24	D6	Oregon, USA	C. Banner	1982	<i>O. kisutch</i>
25	ATCC 33209	Oregon, USA	ATCC	1974	<i>O. tshawytscha</i>
26	MT239	Scotland	D. Bruno*	1993	<i>S. salar</i>

Provided by C. Banner
 * Provided by R. Pascho

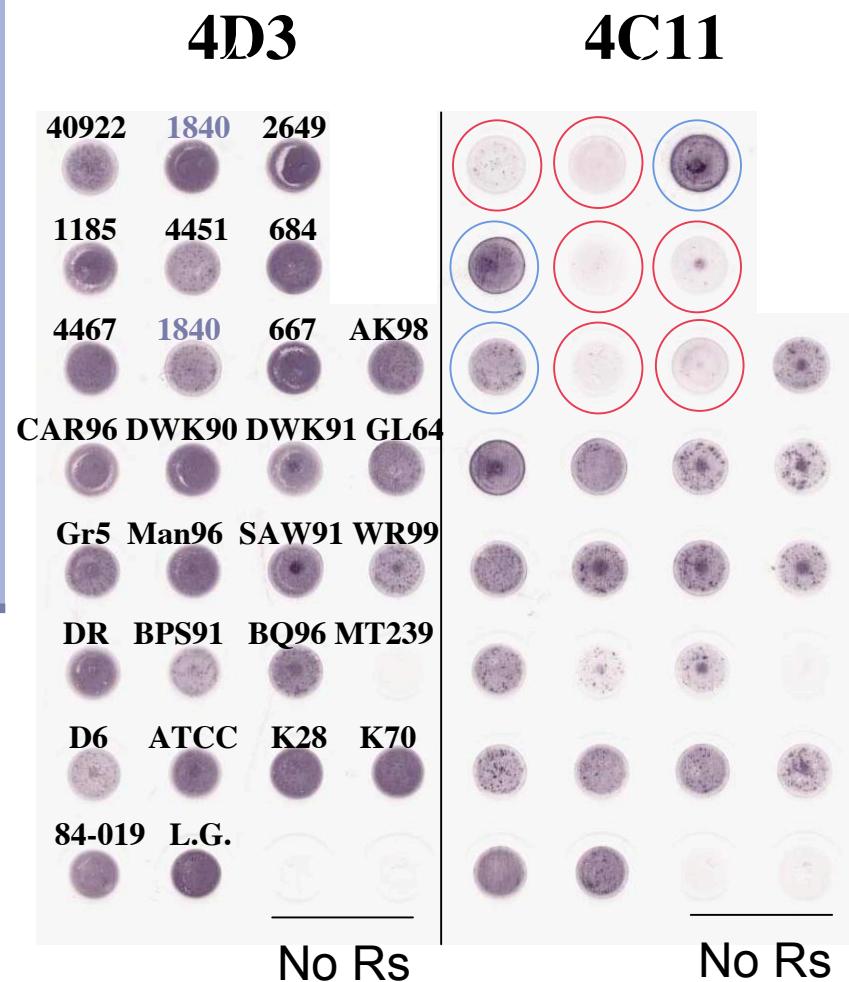
24 isolates, 25 year span

All isolates (except MT239) produce bacterial cell associated p57.



Antigenic diversity in the 4C11 epitope

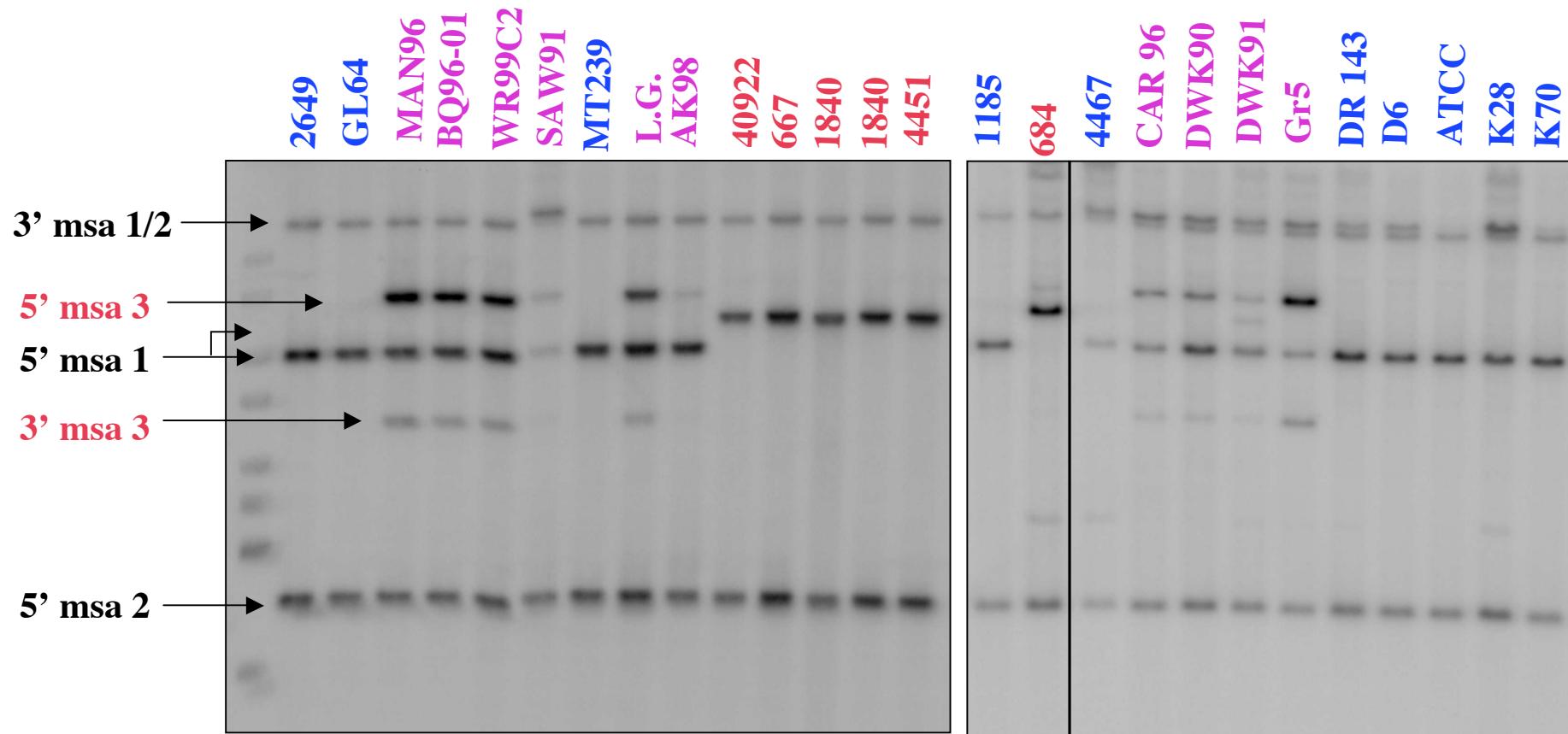
Dot Blot



Norwegian Isolates:
5 reduced 4C11 binding
3 normal 4C11 binding

MT239 (negative control)

Southern blot of *Xho* I digested DNA using a *msa* probe



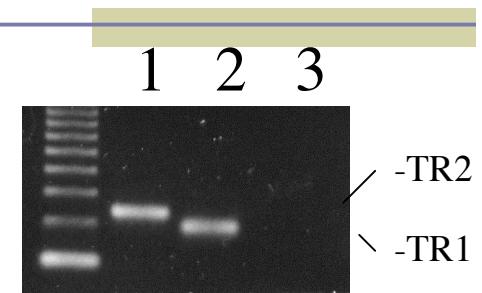
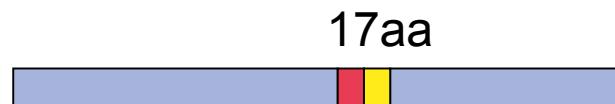
1. All five of the 4C11 epitope-loss mutants from Norway have a shift in 5' *Xho* I *msa1* band. *Msa2* unchanged.
2. Nine isolates are similar to ATCC 33209, including 3 from Norway.
3. Many U.S/Canada isolates appear to have an extra copy(s) of a *msa1*-like gene designated *msa3* (Rhodes et al. 2004)
4. There is variation in the 3' *msa1/2* bands

How do these isolates compare using other molecular markers?

1. ETR-A locus (Grayson et al. 2000)

Exact tandem repeat (51 nt)

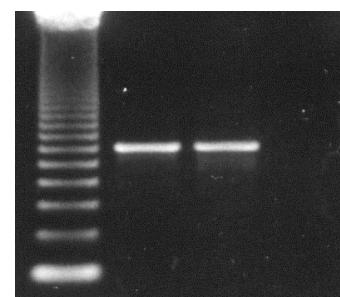
Localized to a 102 kd protein,
cytosolic, unknown function.



2. 16S-23S rRNA intergenic spacer

(Grayson et al. 1999)

4 sequence types (sequevars)

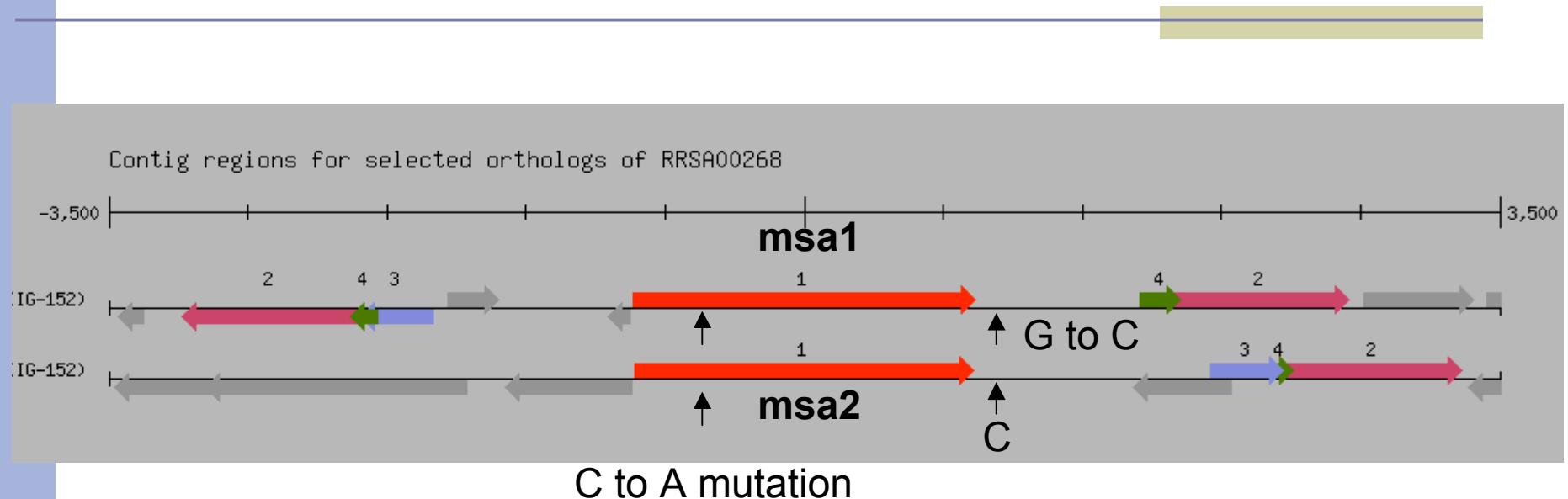


Lanes:

- 1) ATCC 33209
- 2) 684
- 3) control

	Isolate	location	4C11	4D10	msa3	ETRA	16-23s IVS
1	684/87	Aurland, Norway	-	+	-	TR 1	SV4
2	1840/85	Kobbvag, Norway	-	+	-	TR 1	SV4
3	4451/86	Fjordfisk, Norway	-	+	-	TR 1	SV4
4	667/85	Fjordlaks Norway	-	+	-	TR 1	SV4
5	40922/84	Pedro, Norway	-	+	-	TR 1	SV4
6	WR99C2	Washington?, USA	+	+	+	TR 2	SV1
7	CAR 96	Washington, USA	+	+	+	TR 2	SV1
8	Man96	Washington, USA	+	+	+	TR 2	SV1
9	Little Goose	Washington, USA	+	+	+	TR 2	SV1
10	Gr5	Montana, USA	+	+	+	TR 2	SV1
11	SAW91	Idaho, USA	+	+	+	TR 2	SV1
12	DWK90	Idaho, USA	+	+	+	TR 2	SV1
13	DWK91	Idaho, USA	+	+	+	TR 2	SV1
14	AK 98	Alaska, USA	+	+	+	TR 2	SV1
15	BQ96-01	Nanaimo B.C.	+	+	+	TR 2	SV1
16	BPS91-1	Nanaimo B.C.	±	-	+	TR 2	SV1
17	DR 143	Nanaimo, B.C.	+	+	-	TR 2	SV1
18	K28	France	+	+	-	TR 2	SV1
19	K70	England	+	+	-	TR 2	SV1
20	1185/87	Ramsoy, Norway	+	+	-	TR 2	SV1
21	2649/85	Hatlem, Norway	+	+	-	TR 2	SV1
22	4467/86	Aurland, Norway	+	+	-	TR 2	SV1
23	GL64	Lake Michagan	+	+	-	TR 2	SV1
24	D6	Oregon, USA	+	+	-	TR 2	SV1
25	ATCC 33209	Oregon, USA	+	+	-	TR 2	SV1
26	MT239	Scotland	-	-	-	TR 2	SV1

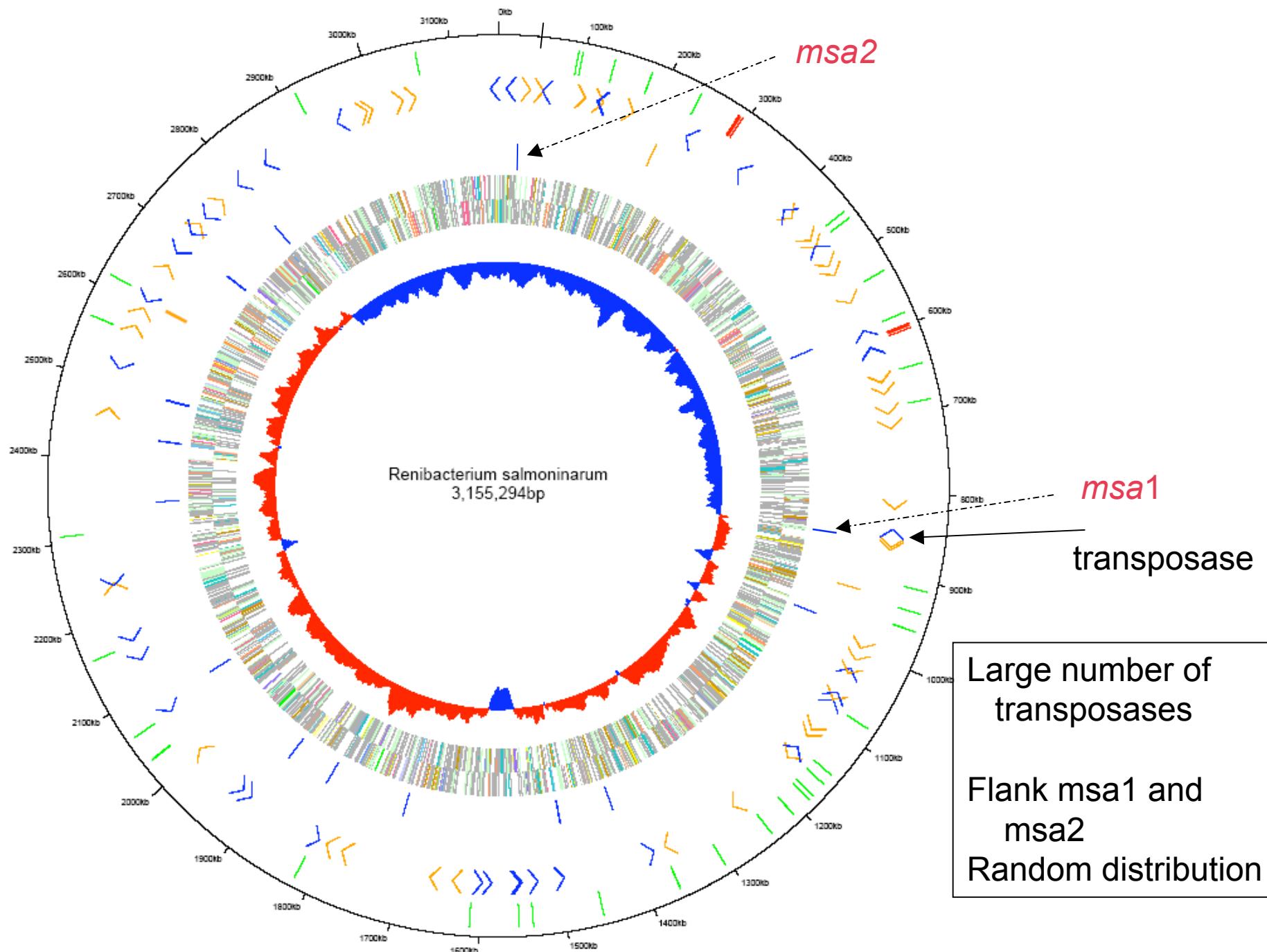
Mechanism of antigenic variation?



Spontaneous mutation in msa2 gene followed by non-reciprocal recombination into msa1?

Recombination with an extrachromosomal element – ie msa3? None of other isolates carry msa3

Role of IS elements?



Summary:

1. Epitopes recognized by 4D3, 4H8, 3H1, and 1A1 are conserved among all isolates tested.
2. Loss of the 4C11 and 4D10 epitopes was observed.
3. Mab 4C11 showed reduced binding to 5 isolates.
4. The 5 Norwegian isolates appear to be genetically similar as they all:
 1. All have 1 Tandem repeat (TR1)
 2. The 16-23S rRNA sequences are SV4.
 3. Shifted *msa1* 5'Xho I band
 4. Do not carry extra *msa3* genes.
5. 4C11⁺ and 4C11⁻ variants can exist at the same location at similar times. *Msa3*⁺ variants seem to be restricted to the U.S./Canada
6. Genome sequence will provide a template for future strain comparisons.

National Center for Cool and Cold Water Aquaculture

Strains

Craig Banner
Ron Pascho

Jennifer Owen



Agricultural Research Service

the in-house research arm of the U.S. Department of Agriculture